CLAIMS

What is claimed is:

1. A method for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of:

providing a Holliday junction structure formed between a target nucleic acid and a reference nucleic acid, the reference nucleic acid differing in sequence from the target nucleic acid in one or more nucleotide positions;

forming a first complex between the Holliday junction structure and a Holliday junction-binder;

contacting the first complex with a receptor for the Holliday junction-binder that specifically recognizes the Holliday junction-binder;

forming a second complex between the first complex and the receptor for the Holliday junction-binder; and

detecting the presence of the Holliday junction structure in the second complex, wherein the presence of the Holliday junction structure in the second complex is indicative of the sequence difference between the target nucleic acid and the reference nucleic acid.

2. The method of claim 1, wherein the target nucleic acid is derived from a targeted region of a test nucleic acid contained in a sample.

3. The method of claim 2, wherein the target nucleic acid is PCR-amplified from a region of the test nucleic acid containing a SNP.

4. The method of claim 2, wherein the test nucleic acid is selected from the group consisting of double-stranded test DNA, single-stranded test DNA, test RNA, test DNA-RNA hybrid of a target gene, chromosome, plasmid, or genome of a biological material.

5. The method of claim 4, wherein the biological material is selected from the group consisting of bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, and humans.

6. The method of claim 2, wherein the sequence of the reference nucleic acid differs from the sequence of the target nucleic acid in a single nucleotide position.

7. The method of claim 1, wherein the target nucleic acid is double-stranded.

8. The method of claim 2, wherein the target nucleic acid comprises a combination of Target-Tail-1 polynucleotide and Target-Tail-2 polynucleotide, wherein the Target-Tail-1 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-1; the Target-Tail-2 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-2; and the sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions.

9. The method of claim 8, wherein the reference nucleic acid comprises a combination of Reference-Tail-1 polynucleotide and Reference-Tail-2 polynucleotide, wherein the Reference-Tail-1 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-1; the Reference-Tail-2 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-2; and the sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions.

10. The method of claim 9, wherein the Target-Tail-1 polynucleotide or the Target-Tail-2 polynucleotide is double-stranded.

11. The method of claim 10, wherein the Reference-Tail-1 polynucleotide or the Reference-Tail-2 polynucleotide is double-stranded.

12. A method for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of:

contacting a target nucleic acid with a reference nucleic acid, the sequence of the reference nucleic acid being the same or differing from the target nucleic acid in one or more nucleotide positions;

subjecting a mixture of the target nucleic acid and the reference nucleic acid to branch migration condition such that a Holliday junction structure forms between the target nucleic acid and the reference nucleic acid when the reference nucleic acid differs in sequence from the target nucleic acid in one or more nucleotide positions;

forming a first complex between the Holliday junction structure and a Holliday junction-binder;

contacting the first complex with a receptor for the Holliday junction-binder that specifically recognizes the Holliday junction-binder;

forming a second complex between the first complex and the receptor for the Holliday junction-binder; and

detecting the presence of the Holliday junction structure in the second complex, wherein the presence of the Holliday junction structure in the second complex is indicative of the sequence difference between the target nucleic acid and the reference nucleic acid.

13. The method of claim 12, wherein the target nucleic acid is derived from a targeted region of a test nucleic acid contained in a sample.

14. The method of claim 13, wherein the target nucleic acid is PCR-amplified from a region of the test nucleic acid containing a SNP.

15. The method of claim 13, wherein the test nucleic acid is selected from the group consisting of double-stranded test DNA, single-stranded test DNA, test RNA, test DNA-RNA hybrid of a target gene, chromosome, plasmid, or genome of a biological material.

16. The method of claim 15, wherein the biological material is selected from the group consisting of bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, and humans.

17. The method of claim 13, wherein the sequence of the reference nucleic acid differs from the sequence of the target nucleic acid in a single nucleotide position.

18. The method of claim 12, wherein the target nucleic acid is double-stranded.

19. The method of claim 13, wherein the target nucleic acid comprises a combination of Target-Tail-1 polynucleotide and Target-Tail-2 polynucleotide, wherein the Target-Tail-1 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-1; the Target-Tail-2 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-2; and the sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions.

20. The method of claim 19, wherein the reference nucleic acid comprises a combination of Reference-Tail-1 polynucleotide and Reference-Tail-2 polynucleotide, wherein the Reference-Tail-1 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-1; the Reference-Tail-2 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-2; and the sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions.

21. The method of claim 20, wherein the Target-Tail-1 polynucleotide or the Target-Tail-2 polynucleotide is double-stranded.

22. The method of claim 21, wherein the Reference-Tail-1 polynucleotide or the Reference-Tail-2 polynucleotide is double-stranded.

23. The method of claim 21, further comprising:

PCR-amplifying the targeted region of the test nucleic acid using

a forward primer for the targeted region of the test nucleic acid,

a first reverse primer for the targeted region of the test nucleic acid

further comprising the sequence of Tail-1, and

a second reverse primer for the targeted region of the test nucleic acid further comprising the sequence of Tail-2.

24. The method of claim 12, wherein the reference nucleic acid or the target nucleic acid has a length of 20-500 nucleotides.

25. The method of claim 12, wherein the reference nucleic acid or the target nucleic acid has a length of 30-100 nucleotides.

26. The method of claim 12, wherein the reference nucleic acid or the target nucleic acid has a length of 50-80 nucleotides.

27. The method of claim 12, wherein the step of detecting the presence of the Holliday junction structure in a second complex includes detecting the presence of one or more strands of the Holliday junction by a method selected from the group consisting of colorimetric detection, fluorescence detection, chemiluminescent detection, enzymatic reaction, gel electrophoresis, mass spectroscopy, and oligonucleotide array.

28. A method for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of:

contacting a target nucleic acid with a reference nucleic acid, the sequence of the reference nucleic acid being the same or differing from the target nucleic acid in one or more nucleotide positions;

subjecting a mixture of the target nucleic acid and the reference nucleic acid to branch migration condition such that a Holliday junction structure forms between the target nucleic acid and the reference nucleic acid when the reference nucleic acid differs in sequence from the target nucleic acid in one or more nucleotide positions;

forming a first complex between the Holliday junction structure and a Holliday junction-binder;

contacting the first complex with a receptor for the Holliday junction-binder that specifically recognizes the Holliday junction-binder, the receptor being immobilized to a substrate;

forming a second complex between the first complex and the receptor for the Holliday junction-binder; and

detecting the presence of the Holliday junction structure in the second complex, wherein the presence of the Holliday junction structure in the second complex is indicative of the sequence difference between the target nucleic acid and the reference nucleic acid.

29. The method of claim 28, wherein the substrate to which the receptor for Holliday junction-binder is immobilized is a solid support.

30. The method of claim 29, wherein the solid support is selected from the group consisting of a microsphere bead, a magnetic bead, a well of a culture plate, glass, membrane and fabric.

31. The method of claim 28, further comprising the step of:

isolating the second complex before the step of detecting the presence of the Holliday junction structure in the second complex.

32. The method of claim 31, wherein the step of isolating includes a method selected from the group consisting of immunoprecipitation, gel electrophoresis, affinity chromatography, oligonucleotide array and flowing fluid sorting.

33. The method of claim 31, wherein the step of detecting the presence of the Holliday junction structure in the isolated second complex includes detecting the presence of one or more strands of the Holliday junction by a method selected from the group consisting of colorimetric detection, fluorescence detection, chemiluminescent detection, enzymatic reaction, gel electrophoresis, mass spectroscopy, and oligonucleotide array.

34. A method for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of:

contacting a target nucleic acid labeled with a tag with a reference nucleic acid, the sequence of the reference nucleic acid being the same or differing from the target nucleic acid in one or more nucleotide positions;

subjecting a mixture of the target nucleic acid and the reference nucleic acid to branch migration condition such that a Holliday junction structure forms between the target nucleic acid and the reference nucleic acid when the reference nucleic acid differs in sequence from the target nucleic acid in one or more nucleotide positions;

forming a first complex between the Holliday junction structure and a Holliday junction-binder;

contacting the first complex with a receptor for the Holliday junction-binder that specifically recognizes the Holliday junction-binder;

forming a second complex between the first complex and the receptor for the Holliday junction-binder; and

detecting the presence of the tag on the target nucleic acid in the Holliday junction structure in the second complex, wherein the presence of the tag on the target nucleic acid in the Holliday junction structure in the second complex is indicative of the sequence difference between the target nucleic acid and the reference nucleic acid.

35. The method of claim 34, wherein the tag is selected from the group consisting of biotin, digoxigenin, fluorescent molecule, chemiluminescent moiety, coenzyme, enzyme substrate, radio isotopes, a particle, nucleic acid-binding protein, and polynucleotide.

36. The method of claim 34, wherein the receptor for the Holliday junction-binder is immobilized to a solid support.

37. The method of claim 36, wherein the solid support is a well of a culture plate.

38. The method of claim 34, further comprising step of:

isolating the second complex before the step of detecting the presence of the Holliday junction structure in the second complex.

39. The method of claim 38, wherein the step of isolating is performed by using a method selected from the group consisting of immunoprecipitation, gel electrophoresis, affinity chromatography, oligonucleotide array and flowing fluid sorting.

40. A method for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of:

contacting a target nucleic acid with a reference nucleic acid, the sequence of the reference nucleic acid being the same or differing from the target nucleic acid in one or more nucleotide positions;

subjecting a mixture of the target nucleic acid and the reference nucleic acid to branch migration condition such that a Holliday junction structure forms between the target nucleic acid and the reference nucleic acid when the reference nucleic acid differs in sequence from the target nucleic acid in one or more nucleotide positions;

forming a first complex between the Holliday junction structure and a Holliday junction-binder;

labeling one or more strand of the target or reference nucleic acid in the first complex with a tag;

forming a second complex between the first complex and the receptor for the Holliday junction-binder; and

detecting the presence of the tag on the Holliday junction structure in the second complex.

41. The method of claim 40, wherein the tag is selected from the group consisting of biotin, digoxigenin, fluorescent molecule, chemiluminescent moiety, coenzyme, enzyme substrate, radio isotopes, a particle, nucleic acid-binding protein, and polynucleotide.

42. The method of claim 40, wherein the receptor for the Holliday junction-binder is immobilized to a solid support.

43. The method of claim 42, wherein the solid support is a well of a culture plate.

44. The method of claim 40, further comprising step of:

isolating the second complex before the step of detecting the presence of the Holliday junction structure in the second complex.

45. The method of claim 44, wherein the step of isolating is performed by using a method selected from the group consisting of immunoprecipitation, gel electrophoresis, affinity chromatography, oligonucleotide array and flowing fluid sorting.

46. A method for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of:

contacting a target nucleic acid with a reference nucleic acid, the sequence of the reference nucleic acid being the same or differing from the target nucleic acid in one or more nucleotide positions;

subjecting a mixture of the target nucleic acid and the reference nucleic acid to branch migration condition such that a Holliday junction structure forms between the target nucleic acid and the reference nucleic acid when the reference nucleic acid differs in sequence from the target nucleic acid in one or more nucleotide positions;

forming a first complex between the Holliday junction structure and a Holliday junction-binder;

contacting the first complex with a receptor for the Holliday junction-binder that specifically recognizes the Holliday junction-binder;

forming a second complex between the first complex and the receptor for the Holliday junction-binder;

labeling one or more strand of the target or reference nucleic acid in the second complex with a tag; and

detecting the presence of the tag on the Holliday junction structure in the second complex.

47. The method of claim 46, wherein the tag is selected from the group consisting of biotin, digoxigenin, fluorescent molecule, chemiluminescent moiety, coenzyme, enzyme substrate, radio isotopes, a particle, nucleic acid-binding protein, and polynucleotide.

48. A method for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of:

contacting a target nucleic acid labeled with a tag with a reference nucleic acid, the sequence of the reference nucleic acid being the same or differing from the target nucleic acid in one or more nucleotide positions;

subjecting a mixture of the target nucleic acid and the reference nucleic acid to branch migration condition such that a Holliday junction structure forms between the target nucleic acid and the reference nucleic acid when the reference nucleic acid differs in sequence from the target nucleic acid in one or more nucleotide positions;

forming a first complex between the Holliday junction structure and a protein that specifically recognizes a Holliday junction;

contacting the first complex with an antibody that specifically binds to the protein that specifically recognizes a Holliday junction;

forming a second complex between the first complex and the antibody; and

detecting the presence of the tag on the target nucleic acid in the Holliday junction structure in the second complex, wherein the presence of the tag on the target nucleic acid in the Holliday junction structure in the second complex is indicative of the sequence difference between the target nucleic acid and the reference nucleic acid.

49. The method of claim 48, wherein the target nucleic acid is derived from a targeted region of a test nucleic acid contained in a sample.

50. The method of claim 49, wherein the target nucleic acid is PCR-amplified from a region of the test nucleic acid containing a SNP.

51. The method of claim 49, wherein the test nucleic acid is selected from the group consisting of double-stranded test DNA, single-stranded test DNA, test RNA, test DNA-RNA hybrid of a target gene, chromosome, plasmid, or genome of a biological material.

52. The method of claim 51, wherein the biological material is selected from the group consisting of bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, and humans.

53. The method of claim 49, wherein the sequence of the reference nucleic acid differs from the sequence of the target nucleic acid in a single nucleotide position.

54. The method of claim 48, wherein the target nucleic acid is double-stranded.

55. The method of claim 49, wherein the target nucleic acid comprises a combination of Target-Tail-1 polynucleotide and Target-Tail-2 polynucleotide, wherein the Target-Tail-1 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-1; the Target-Tail-2 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-2; and the sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions.

56. The method of claim 55, wherein the reference nucleic acid comprises a combination of Reference-Tail-1 polynucleotide and Reference-Tail-2 polynucleotide, wherein the Reference-Tail-1 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-1; the Reference-Tail-2 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-2; and the sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions.

57. The method of claim 56, wherein the Target-Tail-1 polynucleotide or the Target-Tail-2 polynucleotide is double-stranded.

58. The method of claim 57, wherein the Reference-Tail-1 polynucleotide or the Reference-Tail-2 polynucleotide is double-stranded.

59. The method of claim 58, further comprising:

PCR-amplifying the targeted region of the test nucleic acid using

a forward primer for the targeted region of the test nucleic acid,

a first reverse primer for the targeted region of the test nucleic acid

further comprising the sequence of Tail-1, and

a second reverse primer for the targeted region of the test nucleic acid further comprising the sequence of Tail-2.

60. The method of claim 48, wherein the reference nucleic acid or the target nucleic acid has a length of 20-500 nucleotides.

61. The method of claim 48, wherein the reference nucleic acid or the target nucleic acid has a length of 30-100 nucleotides.

62. The method of claim 48, wherein the reference nucleic acid or the target nucleic acid has a length of 50-80 nucleotides.

63. The method of claim 48, wherein the step of detecting the presence of the Holliday junction structure in a second complex includes detecting the presence of one or more strands of the Holliday junction by a method selected from the group consisting of colorimetric detection, fluorescence detection, chemiluminescent detection, enzymatic reaction, gel electrophoresis, mass spectroscopy, and oligonucleotide array.

64. The method of claim 48, wherein the tag is selected from the group consisting of biotin, digoxigenin, fluorescent molecule, chemiluminescent moiety, coenzyme, enzyme substrate, radio isotopes, a particle, nucleic acid-binding protein, and polynucleotide.

65. The method of claim 48, wherein the antibody that specifically binds to the protein that specifically recognizes a Holliday junction is immobilized to a solid support.

66. The method of claim 65, wherein the solid support is a well of a microplate.

67. The method of claim 65, wherein the microplate is a 96-well plate.

68. The method of claim 48, wherein the tag is biotin and the method further comprises:

contacting the second complex with an agent that comprises streptavidin conjugated to an enzyme.

69. The method of claim 68, wherein the enzyme is selected from the group consisting of alkaline phosphatase, perioxidase, and urease.

70. The method of claim 48, wherein the protein that specifically recognizes a Holliday junction is selected from the group consisting of RuvA, RuvC, RuvB, RusA, RuvG, Cce1 and spCce1 from yeast, and Hjc from Pyrococcus furiosusa.

71. The method of claim 48, wherein the protein that specifically recognizes a Holliday junction is a resolvase or recombinase.

72. The method of claim 48, wherein the protein that specifically recognizes a Holliday junction is a recombinant resolvase or recombinase conjugated or fused with a His-tag.

73. The method of claim 48, wherein the antibody is a monoclonal, polyclonal, Fab, fragments of the variable regions, single-chain antibody, or antibody contained in anti-serum.

74. A kit for detecting nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, the kit comprising:

a reference nucleic acid;

forward and reverse target primers for amplifying a targeted region in a test nucleic acid to generate a target nucleic acid;

a Holliday junction-binder; and

a receptor for the Holliday junction-binder.

75. The kit of claim 74, further comprising:

instructions for how to use the kit to detect mutation or nucleotide variation in a sample containing the test nucleic acid.

76. The kit of claim 74, wherein the receptor for the Holliday junction-binder is an antibody that is attached to a solid support.

77. The kit of claim 76, wherein the solid support is a bead, a well of a culture plate, or a membrane.

78. The kit of claim 74, wherein the target nucleic acid or reference nucleic acid is labeled with biotin, and the kit further comprises streptavidin conjugated to an enzyme.

79. The kit of claim 78, wherein the enzyme is selected from the group consisting of alkaline phosphatase, perioxidase, and urease.

80. The kit off claim 74, wherein the reference nucleic acid comprises a combination of Reference-Tail-1 polynucleotide and Reference-Tail-2 polynucleotide, wherein the Reference-Tail-1 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-1; the Reference-Tail-2 polynucleotide comprises a first region that is substantially homologous to the target region of the test nucleic acid and a second region designated as Tail-2; and the sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions.

81. The kit of claim 80, wherein the Reference-Tail-1 polynucleotide or the Reference-Tail-2 polynucleotide is double-stranded.

82. The kit of claim 80, wherein the Reference-Tail-1 polynucleotide or the Reference-Tail-2 polynucleotide is single-stranded.

83. The kit of claim 80, wherein the reverse primer comprises a combination of:

a first reverse primer for the targeted region of the test nucleic acid further comprising the sequence of Tail-1, and

a second reverse primer for the targeted region of the test nucleic acid further comprising the sequence of Tail-2.